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Milestones in contraceptive vaccines development and hurdles in their application

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Abbreviations: aa, amino acids; BSA, bovine serum albumin; β-hCG, beta subunit of human chorionic gonadotropin; β-oLH, beta subunit of ovine luteinizing hormone; CatSper1, cation channels of sperm-1; CRISP1, cystein-rich secretory protein-1; CTP, carboxy terminus peptide; DT, diphtheria toxoid; FA-1, fertilization antigen-1; FSH, follicle stimulating hormone; GnRH, gonadotropin releasing hormone; hCG, human chorionic gonadotropin; KLH, keyhole limpet hemocyanin; LDH-C_4, isozyme of lactate dehydrogenase made up of homotetramer of C-subunits; LH, luteinizing hormone; MAbs, monoclonal antibodies; oFSH, ovine follicle stimulating hormone; oLH, ovine luteinizing hormone; TSH, thyroid stimulating hormone; TT, tetanus toxoid; WHO, World Health Organization; ZP, zona pellucida; ZP1, zona pellucida glycoprotein-1; ZP2, zona pellucida glycoprotein-2; ZP3, zona pellucida glycoprotein-3; ZP4, zona pellucida glycoprotein-4

Contraceptive vaccines have been proposed for controlling the growing human population and wildlife population management. Multiple targets such as gonadotropin releasing hormone (GnRH), luteinizing hormone, follicle stimulating hormone, gonadotropin receptors, sperm-specific proteins and zona pellucida glycoproteins have been exploited to develop contraceptive vaccine and their efficacy investigated and shown in various experimental animal models. Vaccines based on GnRH have found application in immuno-castration of male pigs for prevention of boar-taint. Vaccines based on zona pellucida glycoproteins have shown promising results for population management of wild horses and white-tailed deer. Phase II clinical trials in women with β-human chorionic gonadotropin (β-hCG)-based contraceptive vaccine established proof of principle that these can be developed for human application. Block in fertility by β-hCG contraceptive vaccine was reversible. Further research inputs are required to establish the safety of contraceptive vaccines, improve their immunogenicity and to develop novel vaccine delivery platforms for providing long lasting immunity.

Introduction

Vaccines have been used traditionally to impart protective immunity against various infectious diseases such as diphtheria, pertusis, tetanus, poliomyelitis, measles, hepatitis etc. Further, there are efforts to develop therapeutic vaccines for the treatment of cancers, autoimmune disorders and other diseases.[1,2] For example, to treat asymptomatic or minimally symptomatic metastatic hormone-refractory prostate cancer, Sipuleucel-T was approved by the US Food and Drug Administration (FDA) on April 20, 2010. It is being manufactured by Dendreon Corporation, Seattle, USA and basically involves isolation of patient's dendritic cells, incubation of cells with prostatic acid phosphatase and granulocyte-macrophage colony stimulation factor (GM-CSF) and infusion of activated antigen-presenting cells in the patient.[3] Vaccines meant for autoimmune disorders such as Chrohn's disease, rheumatoid arthritis, type-1 diabetes etc typically involves vaccination with well characterized and defined self-antigenic proteins or peptides to generate tolerance against the protein in question.[2] As an example, synthetic peptide mimicking functional site of matrix metaloprotein (MMP), tricks the immune system of the host to generate 'metallobodies' that block the activity of MMP-2 and MMP-9 and has been proposed for the treatment of inflammatory bowel disease.[4]

The increasing human population, particularly in several developing countries of Asia and Africa, has severe consequences on depletion of natural resources, scarcity of drinking water, threatening food security, in addition to its impact on the environment. In spite of the introduction of steroid hormones based oral contraceptive pills (introduced in 1955), implants, intrauterine devices, male and female condoms, and surgical interventions such as tubectomy and vasectomy, world human population (crossed 7 billion by November, 2011) is still growing (http://www.worldometers.info/world-population/). It is projected that human population may cross 9 billion by 2046. For effective management of human population, scientists have been working on the feasibility of developing vaccine for contraception. Such a vaccine entails generating either humoral and/or cell-mediated immune response against hormones/proteins that have critical role during reproduction. Immune response thus elicited will neutralize their biological activity leading to block of fertility. The feasibility of developing contraceptive vaccine was supported by nature's experiments wherein the presence of naturally occurring auto-antibodies against sperm, zona

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pellucida (ZP) and follicle stimulating hormone (FSH) in women and men were associated with idiopathic infertility.[5-8] In addition to growing human population, there is uncontrolled increase in the population of some animal species. For example, increasing population of elephants in Africa and Kangaroos in Australia, is leading to an increasing conflict for habitation between humans and wildlife species. Further, wild animals may act as vectors or reservoirs for various diseases of zoonotic importance. Globally, zoonoses are said to account for approximately 60% of all infectious disease pathogens (http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/Zoonoses). For example, dogs are one of the main vectors harboring rabies virus, which is transmitted to humans by rabid dog bite. Surgical sterilization such as spaying of female dogs and castration of male dogs have failed to control population of stray/homeless dogs in several developing countries. As a consequence of this, rabies infection is prevalent in these countries with significant human mortality.[9] It is likely that the contraceptive vaccines may provide viable and humane strategy for the management of wildlife population.

Contraceptive vaccines can be broadly categorized into three groups. One group of the vaccines aims to inhibit production of gametes (spermatozoa and egg). These vaccines work by immune-mediated neutralization of gonadotropin releasing hormone (GnRH) secreted by hypothalamus. GnRH plays a critical role in both male and female reproductive system by acting on the anterior lobe of pituitary gland, leading to the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH). FSH plays an important role in the development and maturation of ovarian follicles in females and spermatogenesis in males. LH has an important role in the steroidogenesis in both males and females and LH surge in females is critical for ovulation. Therefore, immune-mediated neutralization of either LH or FSH or generating immune response against their cognitive receptors will also lead to the inhibition of gametogenesis, thus resulting in infertility. Second group of vaccines involves generating immune response against spermatozoa- or oocyte-specific proteins with an aim to interfere with their functions and hence blocking fertilization process. Third group of vaccines target immune-mediated neutralization of human chorionic gonadotropin (hCG). Post-fertilization, hCG synthesized and secreted by growing blastocysts (subsequently placenta), is considered to be critical for the maintenance of conception.[10] Thus, there are multiple targets that can be exploited for the development of contraceptive vaccines. In this review, we aim to discuss the current status of various approaches being used to develop contraceptive vaccines, their merits and limitations for the management of human and wildlife population. Additionally, how the lessons learned from usage of contraceptive vaccines in veterinary applications have helped in refinement of the approach for humans will be described, wherever applicable.

Contraceptive Vaccines Aimed to Inhibit Production of Sperm and Eggs

GnRH, LH, FSH and gonadotropins receptors have been used as immunogens to develop contraceptive vaccines to inhibit production of sperm and oocytes. Their current status is described below:

Contraceptive vaccines based on immune-mediated neutralization of GnRH

GnRH, a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-GlyNH_2) is primarily synthesized and secreted by the hypothalamus.[11] Its presence/synthesis in other tissues such as ovary, testis, prostrate and placenta has also been reported.[12,13] In placenta, GnRH may be involved in the synthesis and secretion of hCG.[14] A variety of GnRH agonists and antagonists have been developed which have great potential to be used for contraception, ovarian stimulation protocol during assisted reproductive technology and treatment of hormone dependent cancers.[15-17] Inhibition of the estrus cycle in female mice, suppression of the estrus in female dogs, prevention of ovulation in rats and abrogation of the pregnancy in mice and baboons (*Papio anubis*) by administration of the murine monoclonal antibodies (MAbs) against GnRH established that GnRH plays a critical role during reproduction.[18-20] To generate immune response against the self proteins, their conjugation with a variety of the carrier proteins such as tetanus toxoid (TT), diphtheria toxoid (DT), keyhole limpet hemocyanin (KLH) and ovalbumin etc has been proposed to provide T-cell help. Therefore, in order to develop contraceptive vaccine based on GnRH, its conjugation with various carrier proteins has been undertaken. Immunization of female rats with GnRH conjugated to bovine serum albumin (BSA) led to anestrous state, decline in LH levels with unimpaired levels of prolactin and reduced size of ovaries having mostly small- to medium-sized follicles.[21] Active immunization of female marmosets monkeys (*Callithrix jacchus*) with GnRH conjugated to TT led to the suppression of cyclicity concomitant with low levels of sex steroid hormones.[22] Immunization of male mice with synthetic vaccine comprising of GnRH linked to muramyl dipeptide through a lysine bridge led to the inhibition of spermatogenesis and decrease in fertility.[23] In an additional approach to develop synthetic vaccine, 7 peptides comprising of GnRH in tandem with different T-helper epitopes of F protein of canine distemper virus were synthesized.[24] Initial active immunization studies in the beagle foxhounds revealed that out of 7 peptides, 5 were highly immunogenic and led to the suppression of testosterone and progesterone. Subsequently, the cocktail of these 5 peptides was able to elicit high antibody titers in five different breeds of dogs.[24]

In addition, attempts have been made to produce GnRH-based contraceptive vaccine by using recombinant DNA technology. Manufacture of recombinant vaccine will be cost-effective as compared with chemically conjugated GnRH-based vaccine. Further, recombinant vaccines may have less batch-to-batch variability as compared with chemically conjugated vaccines. To overcome poor immunogenicity of small self-peptide, recombinant protein comprising of 12 copies of GnRH and receptor-binding domain of Pseudomonas exotoxin A has been expressed in *E. coli*.[25] Immunization of female rabbits with the recombinant protein led to generation of high anti-GnRH antibody titers. The antibodies thus generated, neutralized in-vivo GnRH activity as evident by the presence of degenerated

ovaries in the immunized animals.[25] In another study, cDNA encoding recombinant proteins comprising of either single or 3 copies of GnRH linked with an 8 amino acid (aa) hinge fragment of human IgG_1 and a T helper peptide of measles virus protein was made.[26] The cDNA encoding this complex was further fused to the C-terminus fragment (aa residues 199–326) of asparaginase and the protein was expressed in *E. coli*. The purified recombinant proteins were released from the respective fusion protein by cleavage with hydrochloric acid and further oxidized to make double chain mini protein. Immunization of rats with recombinant protein corresponding to 3 copies of GnRH (GnRH3-hinged-MVP) generated higher GnRH specific antibody response as compared with the recombinant protein having only 1 copy of GnRH (GnRH-hinge-MVP).[26] These investigators extended their studies by further conjugating GnRH3-hinge-MVP recombinant protein with recombinant heat shock protein 65 (Hsp 65) of *Mycobacterium bovis*.[27] Immunization of both male and female rats with the above protein, previously primed with *Bacillus Chalmette-Guerin* (BCG), led to infertility. In male rats, atrophy of the seminiferous tubules and diminished spermatogenesis was observed. In female rats, immunization with this vaccine led to decrease in the size of uteri and reduced ovarian follicular development.[27]

Recombinant GnRH-based vaccine comprising of 5 repeats of GnRH interspersed by 4 T non-B cell epitopes corresponding to circumsporozoite protein of *Plasmodium falciparum*, TT, respiratory syncytial virus, and measles virus has also been expressed in *E. coli*.[28] In addition, the same group has also made another recombinant protein in *E. coli* with 5 copies of GnRH and an additional T-cell epitope of *Mycobacterium tuberculosis*.[29] Immunization of rats with both the above recombinant proteins led to the generation of high titer of anti-GnRH antibodies, decline in testosterone levels to castration levels and atrophy of the prostrate.[29] Immunization of male dogs with *E. coli*-expressed recombinant protein encompassing GnRH and T helper cell epitope p35 of canine distemper virus F protein also inhibited spermatogensis.[30]

Veterinary applications of GnRH-based contraceptive vaccine

In view of the above scientific observations, GnRH-based contraceptive vaccine may be useful for the management of the wildlife population such as white-tailed deer (**Table 1**), dogs and cats and may prove to be a good alternative to surgical sterilization.[31-37] Pre- and post-pubertal rams and boars accumulate androgen derivatives namely androstenone and skatole in their adipose tissues, which gives an unpleasant odor to meat. Therefore, castration of male pigs is routinely performed in order to prevent the occurrence of the boar taint. To avoid surgical castration, immunological castration by employing GnRH-based contraceptive vaccine has also been proposed to remove the pig taint (**Table 1**).[38,39]

Contraceptive vaccines based on immune-mediated neutralization of LH

Keeping in view that LH also plays an important role in reproduction (steriodogenesis in both sexes and ovulation in females), it has also been investigated as a candidate for developing contraceptive vaccine. LH, FSH, hCG and thyroid stimulating hormones (TSH) are composed of two subunits; α-subunit is common in all the four hormones. The β-subunit imparts specificity to these hormones. Active immunization of female rhesus monkeys with the β-subunit of ovine LH (β-oLH) led to inhibition of fertility, which was accompanied by reduced progesterone levels during luteal phase.[40] The anti-fertility effect mediated by active immunization with β-oLH could be reversed by administration of medroxyprogesterone acetate.[41] In addition to LH, the potential of LH-receptor has also been studied. Active immunization of prepubertal male mice with baculovirus-expressed recombinant porcine LH receptor proteins corresponding to either 1–297 aa or 1–370 aa resulted in the decrease of testosterone levels and spermatogenesis.[42] The fertility of the immunized mice was reduced up to 75%.

Contraceptive vaccines based on immune-mediated neutralization of FSH

FSH plays a crucial role in the development of ovarian follicles in females and seminiferous tubules and spermatogenesis in males. The importance of FSH during spermatogenesis is evident by two different observations; (1) Administration of FSH antibodies in male bonnet monkeys (*Macaca radiata*) led to decline in spermatogenesis and thereby fertility;[43] (2) FSH receptor knocked out (FORKO) male mice have underdeveloped testes, reduced serum testosterone levels and spermatogenesis and thus have lower fecundity.[44] Active immunization of male rhesus monkeys with ovine FSH (oFSH) led to decrease in spermatogenesis, without any adverse effect on the serum testosterone levels.[45] However, after two years, in spite of high anti-FSH antibody titers, immunized animals showed return of spermatogenesis with sperm count in the normal range. The sperm morphology, motility and their ability to penetrate zona-free hamster eggs was normal.[45] In another study, active immunization of male bonnet monkeys with oFSH led to a significant decrease in the sperm count in the semen ejaculate, within 150 d post-immunization.[46] Immunization with oFSH had no significant effect on testosterone levels and animals had normal libido. The immunized male monkeys when used for mating with normal females, failed to impregnate suggesting that this immunization procedure led to infertility. The block in fertility was reversible as concomitant with the decline in the anti-oFSH antibody levels, animals regained fertility.[47] Active immunization with oFSH did not lead to any side effects.[47]

Keeping in view the encouraging observations in non-human primates, the group headed by Prof N. R. Moudgal, Indian Institute of Science, Bangalore, India undertook a pilot study in humans, wherein 5 men were immunized with oFSH (**Table 2**).[48] Anti-oFSH antibodies reacted with human FSH (hFSH) as determined by their binding to ^{125}I-labeled hFSH in a radioimmunoassay. Bio-neutralization activity of the immune sera was determined by using sheep testicular receptor binding inhibition assay, which basically involved incubation of particulate sheep testicular FSH receptor with ^{125}I-FSH in presence of immune serum samples.[49] To determine human spermatozoa fertilizing ability, an in-vitro sperm binding and penetration assay employing zona-free hamster eggs has also been developed.[50] The assay has an advantage as it avoids the ethical

Table 1. Contraceptive vaccines for wildlife population management and their relevance for human contraception

Target species	Vaccine formulations	Observations	Relevance for human contraceptive vaccine
Male pigs	GnRH-based contraceptive vaccines; Improvac®	Immunization of male pigs led to immuno-castration and improvement in the meat quality.	Observed inhibition of gonadotropins, sex steroid hormones, atrophy of testes and prostate, contraindicate its use in humans
Wild horses (*Equus caballus*)	Native porcine ZP proteins	Used to manage the population of wild horses at Assateague Island National Seashore, MD, USA.	Safety of long-term vaccination encourage exploration of ZP-based contraceptive vaccine for humans
White-tailed deer (*Odocoileus virginianus*)	GnRH-based contraceptive vaccines; GonaCon™ Native porcine ZP proteins	Active immunization led to reduction in their fawning rates Used to manage the population of white-tailed deer inhabiting Fire Island National Seashore, NY, USA	GnRH-based contraceptive vaccine are not proposed for humans due to disturbed hormonal profile For humans, ZP-based immunogens should be devoid of oophoritogenic epitopes
African elephant (*Loxodonta africana*)	Native porcine ZP proteins	Inhibition of fertility in the immunized female elephants.	Heterologous immunization feasible

and logistic problems associated with the use of human eggs. In some immunized volunteers, a reduction in the sperm count was also observed. No significant changes in the levels of LH, FSH and testosterone were observed in the immunized men. However, a significant reduction in the seminal plasma transferrin was observed.[48]

In addition to FSH, its receptor has also been used as an immunogen to develop contraceptive vaccine. Immunization of male bonnet monkeys with recombinant FSH receptor protein corresponding to aa residues 1–134 of the extracellular domain led to the generation of receptor blocking antibodies.[51] An impairment in the transformation from spermatogonia to primary spermatocytes was observed in the immunized monkeys. Breeding studies revealed that the immunized animals were infertile between 242–368 days of immunization.[51] Filamentous phages displaying mouse LH and FSH receptor decapeptides corresponding to either receptor specific exon 1 (aa residues 18 to 27) or to the homologous exon 4 (aa residues 98 to 107) were engineered.[52] Vaccination of prepubertal BALB/c male mice with engineered phages produced both agonist or antagonist effects leading to reversible contraception. Targeting LH receptor either inhibited or hyperstimulated production of testosterone from leydig cells whereas targeting FSH receptor did not affect testosterone levels.[52] In another study, priming with recombinant hFSH receptor protein (F140) and boosting with a peptide (aa residues 32–44) led to the inhibition of fertility with simultaneous damage to the reproductive organs in male mice.[53] To avoid damage to the reproductive organs but still achieving infertility, priming with the peptide as well as boosting with the same peptide has been proposed.[53] This immunization regimen led to a decrease in fertility, 10 weeks after vaccination. No pathological damage to seminiferous tubules and interstitial cells was observed in peptide prime-boost strategy.[53]

Use of contraceptive vaccines based on GnRH, LH, FSH and gonadotropin receptors in humans: Lessons learned from studies in animals

Immunization with GnRH-based contraceptive vaccine inhibited the secretion of gonadotropins (LH and FSH), sex steroid hormones (testosterone in males; estradiol and progesterone in females) and caused generalized atrophy of reproductive organs (testes, ovaries, prostrate etc). Keeping in view of these undesirable consequences, subsequent to immunization with GnRH, GnRH-based contraceptive vaccine is not being developed for fertility regulation in humans. Similarly, contraceptive vaccines based on LH or its receptor which lead to disturbances in hormonal levels, are also not being pursued as feasible proposition for the management of fertility in humans. The results from initial Phase-I studies on oFSH-based contraceptive vaccine in men are encouraging. However, the potential of FSH and/or its receptor based contraceptive vaccines warrant further investigations to establish a workable proposition for achieving contraception without any untoward side-effects.

Contraceptive Vaccines Based on Sperm and Eggs Specific Proteins

Spermatozoa- and egg-specific proteins that are involved in their development and functions leading to successful fertilization also provide an exciting option to develop contraceptive vaccines.

Spermatozoa-associated proteins

Immunization of female mice or humans with either sperm or their extracts led to the production of anti-sperm antibodies and infertility.[54,55] Though these studies suggested that immunization with sperm can lead to infertility; however, these studies had a drawback of the presence of several proteins that were shared by other somatic cells. It is critical that antibodies developed by contraceptive vaccine based on spermatozoon-specific proteins should not react with any other somatic cells. As a prelude to in-vivo efficacy studies of the contraceptive vaccines based on sperm proteins in suitable animal models, the anti-sperm antibodies may first be evaluated using in-vitro functional assays. The most commonly used in-vitro tests for this purpose are based on studying the effect of anti-sperm antibodies on sperm motility and in-vitro fertilization.[56-59] The effect of anti-sperm antibodies to inhibit in-vitro fertilization is commonly used as a correlate of in-vivo efficacy. In-vitro fertilization assay can be performed by using super-ovulated eggs and capacitated sperm from various species. Depending upon the target sperm antigen being used to propose contraceptive vaccine both zona-denuded as well as zona-encased oocytes has been used. A variety of sperm-specific

Table 2. Clinical trials of contraceptive vaccines in humans

Immunogen	Salient findings	References
oFSH	Immunization of men with oFSH led to generation of bio-neutralizing antibodies. Reduction in sperm count observed in some immunized subjects. No change in LH, testosterone and TSH concentration.	48
CTP of β-hCG (aa residues 109–145) conjugated to DT	Immunization of 30 sterilized women revealed vaccine to be immunogenic and safe.	141
β-hCG-TT	Immunization studies in women revealed vaccine to be immunogenic and safe. Antibodies thus generated neutralized hCG. Women with low antibody titers were not protected from pregnancy.	143, 144, 145, 146
β-hCG-α-oLH-TT/ β-hCG-α-oLH- DT	Phase II clinical trials in fertile women revealed it to be safe and more immunogenic as compared with β-hCG-TT. Only one pregnancy reported from 1224 mating cycles in women having anti-hCG antibody titers above 50 ng/ml. Inhibition of fertility was reversible.	147, 148

proteins have been identified, characterized and their potential to inhibit fertility evaluated in suitable animal models. The characteristics of some of these proteins and their efficacy to inhibit fertility are described below:

LDH-C_4

It is an isozyme of lactate dehydrogenase, made up of homotetramer of C- subunits and is specific to vertebrate spermatozoa.[60] Sub-cellular localization studies suggests its presence in the cytosol of spermatocytes and spermatids, and in the principal piece of spermatozoa. LDH-C_4 null male mice showed normal spermatogenesis and testes development but reduced fertility.[61] Sperm from these mice had lower motility and reduced fertilization capacity. Female mice immunized with mouse LDH-C_4 by intrauterine route developed antibodies against LDH-C_4 and immunized animals showed sub-fertility.[62] Further, male mice immunized systemically with LDH-C_4 also showed reduction in fecundity.[63] Immunogenicity and contraceptive efficacy of LDH-C_4 peptides (human LDH-C_4 aa residues 1–20 and 9–20; and baboon LDH-C_4 aa residues 5–19) either alone or as a chimeric peptide incorporating promiscuous T-cell epitope of TT have been evaluated in rabbits and baboons.[64] The chimeric peptide (baboon LDH-C_4 aa residues 5–19) was more immunogenic and reduced fertility by 62% in the immunized baboons.[64] However, subsequent contraceptive efficacy studies with the same chimeric peptide (baboon LDH-C_4 aa residues 5–19) in female cynomolgus macaque did not yield the desirable contraceptive efficacy.[65]

PH20

It is present on the guinea pig sperm and is involved in the adhesion of the spermatozoa to the zona pellucida (ZP) matrix. Gonad specific expression of PH20 has also been documented in humans and cynomolgus monkeys etc.[66] Active immunization of the male and female guinea pigs with PH20 led to 100% contraceptive efficacy, which was reversible.[67]

Fertilin (PH30)

Fertilin is composed of α (791 aa residues) and β (735 aa residues) subunits in mice. In humans, gene for α subunit is non-functional and β subunit is 735 aa long. Besides, fertilin has also been found in cynomolgus monkeys, rats, guinea pigs, rabbits, pigs, etc. It is present on the sperm surface and plays an important role during sperm adhesion to oocyte and subsequent fusion with the egg plasma membrane (oolemma). Immunization of male guinea pigs with purified guinea pig fertilin resulted in complete infertility, whereas immunization of female guinea pigs resulted only in partial infertility.[68] On the contrary, immunization of male and female European rabbits (*Oryctolagus cuniculus*) with *E. coli*-expressed recombinant α or β-fertilin led to partial inhibition of fertility.[69] Only 4 out of 33 immunized female rabbits failed to conceive.

Sp10

Sp10 from ejaculated human sperm exhibited polymorphism of immunogenic peptides ranging from 18 to 34 kDa.[70] Immunocytochemistry studies revealed its presence on round spermatids and spermatozoa within the adluminal compartment of seminiferous epithelium. Immunofluorescence studies revealed that it cannot be detected on acrosome-intact sperm and is detectable only after acrosome reaction.[70] Recent studies showed that immunization of male mice with recombinant Sp10 resulted in sterility.[71]

Sp17

Sp-17 is a 151 aa residues long polypeptide in humans, while in mice it is composed of 149 aa residues. Localization studies revealed its presence in the cytoplasm of head region of the spermatozoa and throughout the tail region.[72] Earlier this protein was considered to be testis specific, but subsequently its expression has also been documented in several somatic tissues in mice, which however, is much lower as compared with testis.[73] Its expression is enhanced in various types of cancers and may play an important role in cell migration and adhesion. Immunization of female BALB/c mice with synthetic peptide encompassing dominant B-cell epitope of rabbit Sp17 (aa residues 58–66; AEWGAKVDD) and promiscuous T-cell epitope of bovine RNase (aa residues 94–104) led to a dose-dependent reduction in fertility.[74] However, the infertility mediated by this chimeric peptide was mouse strain-specific as no effect on fertility was observed in the immunized B6AF1 mice. It was further demonstrated that induction of peptide-specific T-cell responses and cytokines were major factors in mediating infertility.[74]

Sp56

In mouse, it is a 579 aa long protein, present on plasma membrane of the spermatozoa head region and plays an important role in sperm-egg interaction.[75] Immunization of female BALB/c mice with *E. coli*-expressed recombinant Sp56 fusion protein led to sub-fertility.[76] However, *sp56* null mice showed normal fecundity and thus undermining its role during fertilization.[77]

Fertilization antigen (FA)-1

In mice, this protein is 164 aa long and is shown to interact with zona pellucida glycoprotein-3 (ZP3) in-vitro.[78] It is localized on post-acrosomal region of the spermatozoa and the MAbs against human FA-1 were shown to inhibit bovine in-vitro fertilization.[56] Immunization of mice with recombinant FA-1 led to generation of the spermatozoa/testis-specific antibody response resulting in reversible inhibition of fertility by affecting sperm-zona binding and the fertilization process.[57]

Izumo

It is an immunoglobulin superfamily protein and plays an important role in fusion of sperm membrane with oolemma. In humans, this protein is 350 aa long and in mice 397 aa long. It is located on the inner acrosomal membrane of spermatozoa and is accessible to the antibodies only after acrosome reaction. Izumo knockout male mice are sterile but otherwise healthy.[79] Sperm from these animals bind to the zona pellucida, penetrate normally but were incapable of fusing with the eggs.[79] Active immunization studies in mice with the recombinant Ig-like domain of Izumo showed reduction in fertility of immunized animals.[58] In addition to Ig-like domain of Izumo, synthetic peptides corresponding to Izumo (aa residues 166–182, 308–323, 341–359, 371–385) conjugated with different carrier proteins also showed their contraceptive potential during active immunization studies in mice.[80] Interestingly, the contraceptive efficacy of synthetic peptide corresponding to Izumo was enhanced when the animals were co-immunized with synthetic peptides corresponding to FA-1, Sp56 and YLP_{12} conjugated with various carrier proteins.[80]

YLP_{12}

Using the phase display technique, a novel dodecamer sequence (YLPVGGLRRIGG) designated as YLP_{12} was identified that binds to the ZP3-primary sperm receptor.[81] It was localized in the acrosomal region of the human spermatozoa. Active immunization studies with this peptide conjugated with the binding subunit of recombinant cholera toxin led to block in fertility, which was reversible as antibody titers declined.[82] Further, immunization of female mice with virus-like particles (VLPs) derived from Johnson Grass Mosaic Virus coat protein expressing YLP_{12} as a fusion peptide with ZP3 peptide or physical mixture of VLPs presenting either YLP_{12} or ZP3 epitope led to the curtailment of fertility.[83]

CatSper1

Cation channels of sperm (CatSper) have also been explored for their contraceptive potential. There are four members in CatSper family, which form heterotetrameric channels. Mice deficient in any member of the family are completely sterile. These channels are located in the principle piece of the spermatozoa tail and are required for its hyperactivation and motility. Immunization of female mice with synthetic peptides corresponding to predicted B-cell epitopes in the extracellular part of the transmembrane domains and pore region of CatSper1 that share high identity at aa level between mouse and human CatSper1 led to inhibition of fertility.[84] The sperm from immunized animals showed impaired ability to fertilize eggs in-vitro.

Eppin

Eppin stands for epididymal protease inhibitor, which is a serine protease inhibitor present on the surface of entire sperm. It is 134 aa long protein in mice and 133 aa long in humans.[85] Immunization of male bonnet monkeys (*Macaca radiata*) with eppin led to the generation of high antibody titers with concomitant infertility in approximately 78% of the immunized animals.[86] Block in fertility was reversible as 5 out of 7 high-titerd monkeys regained fertility, subsequent to decline in the antibody titers.[86]

80 kDa human sperm antigen

It is a glycoprotein whose expression is confined to male gonads.[87] Immunization with the synthetic peptides (NTRVAGQTVAFL and LFPQYVAYITNLKA) corresponding to 80 kDa Human Sperm Antigen conjugated to KLH led to reversible block of fertility in male rabbits.[88] Immunization with synthetic peptide (LFPQYVAYITNLKA) conjugated to KLH also led to block of fertility in male marmosets.[88]

Proacrosin

It is a serine protease present in acrosome of mammalian sperm. It is 421 aa long in humans and is synthesized as proacrosin, which is processed into active form, acrosin. Proacrosin/acrosin plays an important role in the binding and penetration of the acrosome-reacted sperm through ZP matrix. Active immunization of male mice with DNA vaccine encoding proacrosin led to decrease in fertility of the immunized animals.[59] A decrease in litter size was also observed as compared with the control group. The antibodies thus elicited by immunization with DNA vaccine also led to the inhibition of sperm-zona binding and Ca^{2+}- ionophore induced acrosome reaction.[59]

Cystein-rich secretory protein-1 (CRISP1)

It is an epididymal protein which belongs to CRISP (Cystein-Rich Secretory Proteins) family.[89] Its size in humans is 249 aa and in mice it is 244 aa. *CRISP1* deficient mice showed normal fecundity but sperm showed lesser capability to fuse with egg during in-vitro fertilization.[90] A part of the protein is lost during the capacitation while remaining part migrates from the dorsal side of sperm to the equatorial region after capacitation and acrosome reaction.[90] Immunization of male and female mice with the DNA vaccine encoding mouse CRISP1 showed reduced fertility.[91]

Relevance of sperm-based contraceptive vaccine for use in humans

The above studies showed variable contraceptive efficacy of a variety of spermatozoa-associated proteins in different animal models. These active immunization studies have not revealed any untoward side-effects. One can argue that how critically the side-effects have been examined by various researchers while evaluating the contraceptive potential of sperm-specific proteins. So far, no contraceptive vaccine based on sperm-specific proteins has gone through pre-clinical safety evaluation in animal models

and thus not entered into the Phase-I clinical trials in humans. Further scientific inputs and rigorous investigations are required to propose a candidate contraceptive vaccine based on sperm-specific antigens for use in humans.

Contraceptive vaccines based on zona pellucida glycoproteins

Mammalian egg is surrounded by an extracellular glycoproteinaceous matrix known as zona pellucida (ZP). It plays a critical role in relative species-specific binding of the spermatozoon to the oocyte, induction of the acrosome reaction in the zona bound spermatozoa, prevention of polyspermic fertilization and protection of the growing blastocyst till implantation takes place. In mammals, ZP matrix is composed of either 3 or 4 glycoproteins.[92] In mice, it is composed of 3 glycoproteins designated as ZP glycoprotein-1 (ZP1), -2 (ZP2) and -3 (ZP3). In pigs and dogs, it is also composed of 3 glycoproteins but instead of ZP1, ZP glycoprotein-4 is present. In rats, hamsters, non-human primates and humans, ZP matrix has all the 4 glycoproteins. Structure and functions of all the four ZP glycoproteins during fertilization from various species has been investigated by various groups, which have been reviewed recently.[92] Sequencing of all the four ZP glycoproteins from various species revealed that a given zona protein has variable degree of sequence conservation at the aa level. This property of ZP proteins has made heterologous immunization as a feasible proposition. For example, antibodies generated against porcine ZP3 showed immunological cross-reactivity with human ZP.[93] Active immunization of females using various animal models with either the crude porcine zonae pellucidae or the purified native porcine zona proteins led to the curtailment of fertility.[94-99] Observed infertility was invariably associated with ovarian dystrophy accompanied by follicular attresia. However, use of highly purified native porcine zona proteins supplemented with adjuvants other than complete Freund's adjuvant led to a significant decrease in ovarian pathology.[98-100]

Utility of native ZP-based contraceptive vaccine for wildlife population management

In spite of the observations that immunization with ZP glycoproteins lead to ovarian dystrophy, ZP-based contraceptive vaccines have been found useful in the wildlife population management. Contraceptive vaccine based on porcine ZP has been used for decades to manage population of feral horses (*Equus caballus*) at Assateague Island National Seashore, MD, USA (**Table 1**).[101,102] It was shown that third consecutive annual booster of porcine ZP led to 79% efficacy in preventing pregnancies in mares.[102] In addition to wild horses, porcine ZP based contraceptive vaccine has also been used to control the population of white-tailed deer (*Odocoileus virginianus*) inhabiting Fire Island National Seashore, NY, USA (**Table 1**).[103,104] Between 1993 and 1999, fawning rates among individually known vaccine treated adult female deer decreased by 78.9% from pretreatment rates.[104] The porcine ZP based vaccine was delivered remotely by using dart-gun approach. Long-term follow-up of the porcine ZP immunized wild horses and white-tailed deer did not reveal any significant debilitating health effects.[105-107] The potential of porcine ZP-based contraceptive vaccine to control the population of African elephant (*Loxodonta africana*) has also been explored (**Table 1**).[108]

Potential of recombinant ZP proteins for inhibiting fertility

Use of recombinant ZP proteins as candidate for developing contraceptive vaccines has been proposed to overcome the limited availability of purified native porcine zona proteins from pig oocytes and the possibility of contamination by other ovarian associated proteins. Immunization of female mice with *E. coli*-expressed recombinant porcine ZP3 and ZP4 with or without promiscuous T cell epitopes of either TT or bovine RNase led to the generation of high antibody titers with concomitant decrease in fertility.[109] In general, fusion proteins with promiscuous T cell epitopes generated higher antibody titers as well as contraceptive efficacy as compared with recombinant porcine ZP3 and ZP4 without promiscuous T cell epitope. The litter size in the immunized animals was significantly decreased as compared with the control group.[109] Immunization of female marmoset (*Callithrix jacchus*) with the recombinant human ZP3 expressed in Chinese Hamster Ovary (CHO) cells induced long-term infertility.[110] Immunized animals showed ovarian pathology associated with the depletion of primordial follicles.[110] Immunization of cynomolgus monkeys (*Macaca fascicularis*) and baboon (*Papio cynocephalus*) with various mammalian-expressed recombinant human ZP glycoproteins revealed that those immunized with ZP4 showed higher contraceptive efficacy as compared with those immunized with ZP2 or ZP3.[111] During the period of high antibody titers, some immunized animals experienced disruption of the menstrual cycles, which subsequently returned to normal. Immunization of female baboons (*Papio anubis*) with *E. coli*-expressed recombinant bonnet monkey ZP4 conjugated to DT led to reversible block of fertility.[112] Using homologous animal model, female bonnet monkeys immunized with *E. coli*-expressed recombinant bonnet monkey ZP4 conjugated with DT also led to the generation of high antibody titers against ZP4 as well as DT.[113] Mating of immunized females with males of proven fertility did not result in conception. Long-term follow-up of the immunized female monkeys revealed that they failed to conceive even when the antibodies against ZP4 were not detectable in the serum. Ovarian histopathology revealed the presence of attretic follicles with degenerated oocytes.[113] Reasons for reversible vs. irreversible block of fertility by recombinant bonnet monkey ZP4 coupled to DT in female baboons as compared with bonnet monkeys are not clear at this stage. In addition, immunization of non-descript females dogs with *E. coli*- expressed recombinant dog ZP3 conjugated with DT also led to the curtailment of fertility.[114] Ovarian histology of the immunized dogs revealed degenerative changes in the ZP matrix and follicular attresia. To avoid chemical conjugation of recombinant dog ZP3 with DT, fusion protein encompassing promiscuous T-cell epitope of TT (aa residues 830–844) followed by dilysine linker and dog ZP3 (TT-KK-ZP3) has been expressed in *E. coli*.[115] Further, the protein has been expressed without His_6-tag as presence of His_6-tag may influence immunogenicity and efficacy of a vaccine as shown for recombinant MSP1 protein of *Plasmodium falciparum*.[116] The process for purification of recombinant TT-KK-ZP3 from

inclusion bodies was optimized. Significant reduction in fertility was observed in female mice immunized with the recombinant TT-KK-ZP3.[115] In Australia and New Zealand, the potential of recombinant brushtail possum (*Trichosurus vulpecula*) ZP3 protein to control the fertility of Koalas (*Phascolarctos cinereus*) and Eastern Grey Kangaroos (*Macropus giganteus*) has also been investigated.[117,118]

Inhibition of fertility by DNA vaccine encoding ZP proteins

Immunization of mice with DNA vaccines encoding either bonnet monkey ZP4 or canine ZP3 led to the generation of antibodies reactive with respective native ZP matrix.[119,120] Antibodies generated by DNA vaccine encoding bonnet monkey ZP4 also led to inhibition of the binding of human sperm to human ZP.[119] Antibodies elicited by DNA vaccine encoding chimeric protein comprising the epitopes of human ZP3 and ZP4 led to significant reduction in the acrosome reaction mediated by the recombinant human ZP3 and ZP4 in capacitated human sperm.[121]

The prospect of using DNA vaccine encoding partial sequence of rabbit ZP3 (aa residues 263–415) to inhibit fertility in female mice has been demonstrated.[122] Immunized female mice showed ovarian follicles at different stages of development suggesting that this immunization protocol had no adverse effect on ovarian functions.[122] Co-immunization of female mice with the DNA vaccine encoding mouse ZP3 and recombinant ZP3 led to a significant inhibition of fertility.[123] Ovarian histology revealed normal follicular development, which was associated with reduced T-cell responses. This immunization regimen led to a decrease in the production of inflammatory cytokine and IFN-γ. An increase in the production of IL-10 and Fox P3 in CD4 T cells was also observed suggesting involvement of the T regulatory cells.[123] These studies demonstrated that ZP-based DNA vaccines can elicit bioactive antibodies; however, further investigations are required to propose a practical proposition for controlling population of either humans or animals at the field level.

Live vector-based contraceptive vaccines expressing ZP proteins

In addition to using recombinant zona proteins, attempts have also been made to immunize animals with genetically modified live vectors to generate immune response against zona proteins. Immunization of mice with attenuated *Salmonella typhimurium*-expressing mouse ZP3 by oral route led to the generation of anti-ZP3 antibodies.[124] Immunized animals showed reduction in fertility. Host-specific live vectors expressing zona proteins have also been evaluated for their contraceptive potential. For example, mice infected with recombinant ectromelia virus (a natural pathogen that causes mouse pox) expressing mouse ZP3 were infertile for 5 to 9 mo after infection.[125] Infertility was found to be associated with disruption in ovarian follicular development. Female rabbits infected with recombinant myxoma virus expressing rabbit ZP4 (previously designated as ZPB) showed presence of anti-ZP4 antibodies and infertility accompanied with ovarian pathology.[126,127] Boosting of rabbits infected with recombinant myxoma virus with recombinant rabbit ZP4 led to further curtailment in fertility.[127] Subsequently, recombinant myxoma virus expressing rabbit ZP2 and ZP3 have also been made.[128] Infection of female rabbits with myxoma virus expressing rabbit ZP2 had no effect on fertility, in spite of the generation of anti-ZP2 antibodies. However, animals infected with myxoma virus expressing rabbit ZP3 led to infertility.[128] Disruption in the development of the ovarian follicles was observed from 15 to 40 d post-infection. Subsequently, ovarian follicles development was normal.[128] Infection of mice with recombinant cytomegalovirus (mouse-specific β herpes virus) expressing mouse ZP3 also led to permanent infertility, principally due to induction of the ovarian autoimmune pathology leading to progressive oocyte depletion.[129,130]

The idea of developing host-specific live vector-based contraceptive vaccine to control the population of pests such as wild rats and rabbits in Australia was to release these live vector contraceptive vaccines in the environment so that recombinant virus gets transmitted from one animal to another, thereby leading to effective management of their population. However, one of the limitations of this approach is that recombinant virus has lower infectivity as compared with wild type. Second, environmentalists have expressed concern about the stringency of the host-specificity of viruses and its consequences, if by chance these recombinant viruses loose host specificity. Keeping this in view, Commonwealth Scientific Industrial Research Organization (CISRO), Australia has stopped funding this approach for controlling the population of pests such as wild rats and rabbits.

Lessons learned from animal studies to investigate the feasibility of developing ZP-based vaccine for contraception in humans

The observed ovarian pathology in various animal models, subsequent to immunization with the zona protein-based contraceptive vaccine, is one of the major hurdles in their application for contraception in humans. It was demonstrated that the oophoritis was mediated due to the presence of oophoritogenic T-cell epitope using mouse ZP3 as a model antigen.[131,132] Immunization of mice with B cell epitope of ZP3, segregated from oophoritogenic T cell epitope, led to block in fertility without ovarian pathology.[133] Keeping these observations in view and with an aim to develop safe contraceptive vaccine for application in humans, various groups have mapped B cell epitopes of various zona proteins with the premise that antibodies generated against these immunogens will elicit antibodies blocking sperm-egg interaction and will not lead to ovarian pathology. The MAbs have been used to map the relevant B cell epitopes of various ZP proteins. As an example, MAbs against *E. coli*-expressed recombinant bonnet monkey ZP4, reacting with the ZP of human eggs, have been used to map the B cell epitopes.[134] Before mapping the B cell epitopes recognized by MAbs, it is pertinent to establish the in-vitro contraceptive efficacy of these antibodies. Classically, either Sperm Binding and Penetration Assay or Hemizona Assay have been employed for this purpose.[134,135] To avoid variability in the number of sperm bound per egg, hemizona assay has been increasingly employed by various researchers. The assay basically uses human eggs cut into two halves using micromanipulators, where one half is incubated with the pre-immune or control serum sample and other half

incubated with immune or test serum sample. After incubation, each hemizona is rinsed in fresh medium before exposure to capacitated human sperm. After co-incubation, each hemizona is again rinsed vigorously to detach loosely bound sperm. The number of sperm tightly bound to the outer hemizona surface is counted. The results are expressed as Hemizona Index (HZI) calculated by dividing the number of bound sperm in test serum by number of bound sperm in control serum multiplied by 100. Using immunogens corresponding to defined epitopes, reduction in fertility without concomitant ovarian pathology was observed in white-tailed deer immunized with porcine ZP4 peptide corresponding to aa residues 79–130 and mice immunized with mouse ZP3 peptide corresponding to aa residues 328–442.[136,137] Immunization of female bonnet monkeys with synthetic peptide of bonnet monkeys ZP3 conjugated with DT also led to block of fertility.[138] Immunization had no effect on the ovarian follicular development.[138] These observations suggest that it is feasible to develop ZP-based contraceptive vaccine by employing immunogens corresponding to B cell epitopes and devoid of oophoritogenic T cell epitopes to achieve contraception without oophoritis for eventual use in humans.

Contraceptive Vaccines Based on hCG

The β-subunit of hCG is 145 aa long and as compared with β-hLH, it has a unique extension of 30 aa at C-terminus designated as Carboxy Terminus Peptide (CTP). Both CTP of β-hCG and β-hCG have been used as candidate immunogens to develop immunocontraceptive vaccine for human application.

Contraceptive vaccines based on CTP of β-hCG

Immunization of female baboons with CTP of β-hCG conjugated to DT led to the curtailment of fertility.[139] Subsequently, Phase I clinical trials of birth control vaccine incorporating synthetic CTP (109–145 aa residues) was conducted in 30 sterilized (by tubal ligation) women at Bedford Park, Australia by WHO Task Force on Vaccines for Fertility Regulation (**Table 2**).[140] Follow-up of the immunized women for 6 mo did not reveal any important adverse reactions. In the group of women immunized with the highest dose of vaccine, potentially contraceptive levels of antibodies to hCG were generated.[140] Encouraged by the findings from Phase I clinical trials of CTP of β-hCG based birth control vaccine, WHO initiated Phase II clinical trials in Sweden. However, due to unacceptable adverse reactions in the vaccinated women, the continuation of Phase II clinical trial was abandoned. To enhance the immunogenicity of synthetic CTP vaccine, microsphere formulations based on polylactic-co-glycolic acid incorporating synthetic CTP vaccine comprising promiscuous T-cell epitope of TT co-linearly synthesized with CTP of β-hCG from aa residues 111–145 has been evaluated.[141] Single injection of this formulation in rabbits elicited a strong antibody response with equivalent duration as achieved by 3 injection schedule of the same immunogen delivered in squalene-based water-in-oil emulsion.

Contraceptive vaccines based on β-hCG

In addition to CTP of β-hCG, whole β-hCG conjugated with different carrier proteins as an immunogen for the development of birth control vaccine has also been investigated with the notion that antibodies generated against β-hCG will have better bio-neutralization capacity for hCG as compared with those generated against CTP of β-hCG. After extensive immunogenicity and safety studies in various animals models, including non-human primates, the first prototype vaccine comprising β-hCG linked to TT, underwent Phase I clinical pharmacological trials at two centers in India as well as centers at Helsinki, Uppsala, Bahia and Santiago (**Table 2**).[142-145] These investigations revealed that the vaccine is immunogenic but antibody titers varied among immunized women. The formulation generated low antibody titers in appreciable number of the immunized women. However, immunization with this formulation was safe. Subsequently to increase its immunogenicity, β-hCG was annealed to α-subunit of oLH, which was conjugated with either TT or DT (β-hCG-α-oLH-TT/β-hCG-α-oLH-DT). After establishing its immunogenicity and safety in various animal models, Phase II clinical trials in fertile women were initiated at multiple centers in India (**Table 2**). Active immunization of women with β-hCG-α-oLH-TT/β-hCG-α-oLH-DT led to the generation of hCG neutralizing antibodies.[146,147] The hCG neutralization capacity of antibodies was estimated by Receptor Binding Inhibition Assay.[146] Basically, the assay involved incubation of Wistar rat's testicular homogenate with various dilutions of immunized women serum samples and 1 ng of ^{125}I-labeled hCG for 2 h at 37 °C. Dilution of serum samples resulting in 50% inhibition in the binding of ^{125}I-hCG were computed by regression analysis. Bio-neutralization capacity was expressed in ng of hCG neutralized per ml of the serum. Immunized women with circulating bio-neutralizing antibody titers above 50 ng/ml were protected against conception. Only one pregnancy was observed out of 1224 cycles in women with antibody titers above 50 ng/ml. Block in fertility was reversible, as immunized women conceived when antibody titers declined to less than 35 ng/ml. However, this vaccine formulation with an improved immunogenicity also failed to elicit anti-hCG antibody titers above protective threshold in 100% of the recipients. To overcome dependence on the availability of purified β-hCG from native source (urine from pregnant women) and to further improve immunogenicity, fusion recombinant protein comprising of B-subunit of *E. coli* heat-labile enterotoxin and β-hCG was expressed in *Pichia pastoris*.[148] The recombinant protein adsorbed on Alhydrogel when used along with *Mycobacterium indicus pranii* generated very high anti-hCG antibody titers in 100% of the immunized animal.[148,149] The antibody titers generated in different strains of mice were several fold higher than the protective threshold of 50 ng/ml in women.[149] Further studies are awaited to ascertain the immunogenicity and contraceptive efficacy of this recombinant protein in women.

Strengths and Weaknesses of Contraceptive Vaccines

One of the strengths of the contraceptive vaccines is that the infrastructure to deliver vaccines exists in most developing countries. It is likely that the immunological approaches

for contraception are cost-effective and free from the risk of user's failure. As compared with the vaccines for prevention of infectious diseases, contraceptive vaccines are meant for young and healthy subjects, who have the availability of alternate methods of contraception such as steroid hormones based oral pills and implants, intrauterine devices and male and female condoms. Hence, it is imperative that the contraceptive efficacy achieved by vaccines should be comparable to the above alternate available options. However, β-hCG based contraceptive vaccine, only one that has been evaluated in humans, has not shown contraceptive efficacy that is comparable to any of the other alternative available contraceptive options described above. Second major drawback of the contraceptive vaccines is the variability of immune response among immunized individual subjects. Variability of immune response is not unique to contraceptive vaccines as it is also a common feature with vaccines meant for prevention of infectious diseases. However, the saving factor in the latter is the statistical improbability of every one in a community getting infected whereas every human subject opting for anti-fertility vaccine would be of proven fertility. The observed variability in immune response in the vaccinated women will warrant monitoring of antibody titers at regular periods to arrive at decision about administering booster injection. In the Family Planning Program, it may be a daunting task, especially in the developing countries. Third, it is critical to establish the safety of the contraceptive vaccines beyond doubt. Long-term safety studies over 15 to 20 y including teratological studies should be undertaken, before contraceptive vaccines may be recommended for human use. Contraceptive vaccines based on immune-mediated neutralization of GnRH and LH may have utility only in the control of fertility in animals and are not likely to be safe or acceptable for eventual human application (**Table 3**). Contraceptive vaccines based on either spermatozoa- or egg-specific proteins and aiming to inhibit the process of fertilization rather than gametogenesis would in principle be acceptable for human application. Long-term follow-up studies of the porcine ZP-based contraceptive vaccine immunized feral horses and white-tailed deer revealed no significant deleterious effects on the health of the immunized animals except oophoritis.[105-107] Follow-up of the immunized feral horses between 1990 to 2002 revealed no significant effect of porcine ZP contraception on the season of birth or foal survival as compared with unvaccinated feral horses habitating Assateague Island National Seashore, Maryland, USA.[106] The block in fertility was reversible.[105] Subsequent fertility was not affected in the female offspring born to vaccinated mothers.[105] White-tailed deer vaccinated with porcine ZP-based contraceptive vaccine showed ovarian pathology representing eosinophilic oophoritis.[107] Thus, the vaccines based on gamete-specific antigens require extensive safety studies in various experimental animal models before these can be considered for even Phase I safety and immunogenicity studies in human subjects (**Table 3**). ZP-based immunogens should not induce oophoritis. Among the two contraceptive vaccines, one based on oFSH that has undergone Phase I trials in men and the second based on β-hCG which has gone through Phase II efficacy studies, the latter seems to have higher probability of clinical application (**Table 3**).

Major Challenges in the Application of Contraceptive Vaccines

One of the common denominators, whether the contraceptive vaccines are meant for humans or wildlife population management, is to improve their immunogenicity. Further, the immune response elicited by contraceptive vaccines should be long lasting so as to achieve contraceptive efficacy of at least one year. It is desirable that the infertility mediated by contraceptive vaccine in humans is reversible. In the context of wildlife population management, it will be ideal, if single injection of contraceptive vaccine generates

Table 3. Strength and weakness of various contraceptive vaccines

Target	Strength	Weakness	Clinical application
GnRH	Workable in both males and females. Contraceptive efficacy demonstrated in animals	Inhibit secretion of gonadotropins, sex steroid hormones and atrophy of reproductive organs	Useful for veterinary application. Not likely to be used in humans
LH	Contraceptive efficacy evaluated in non-human primates	Disturbance in steroid hormonal profile	Not being pursued for veterinary or human use
FSH	Extensive immunization studies in non-human primates. Phase I clinical studies in men	Discrepant results on the efficacy to decrease spermatogenesis in non-human primates	Additional safety and efficacy studies needed
Sperm-specific proteins	Several sperm-specific proteins documented and contraceptive efficacy established in animals	Long-term safety studies not performed	Good candidate for development of contraceptive vaccine for humans
Zona pellucida	Contraceptive efficacy demonstrated in animals. Long-term follow-up of immunized animals showed no debilitating effect on health	Generate oophoritis	Useful for veterinary application. Immunogen design not eliciting oophoritis critical for human use
hCG	Safety studies performed. Demonstrated contraceptive efficacy in women. Block in fertility reversible	Inadequate protective antibody titers in all vaccinated women. Variability in immune response among women	Good candidate for developing contraceptive vaccine for humans

adequate antibody titers leading to permanent sterility. Thus, it is imperative to develop more potent adjuvants and novel vaccine delivery platforms. Additionally, the safety of the contraceptive vaccines that entails generating immune response against the self protein(s) needs to be established beyond doubt. Both in-vitro and in-vivo experimental model systems should be developed to establish safety of contraceptive vaccines before clinical trials are initiated in humans. In addition, the adverse effects on the health of the progeny born to vaccinated human subjects in cases of vaccine failure should be addressed. Attempts have been made to address this issue in women immunized with β-hCG based contraceptive vaccine. The anti-fertility effect of the vaccine was reversible as women with low anti-hCG antibody titers conceived and pregnancy progressed to term.[150] Children born showed normal early developmental parameters.[150] However, additional safety studies need to be pursued more rigorously and for longer duration of say 20–25 y, before contraceptive vaccine can be recommended for fertility regulation in humans. Delivery of vaccine to free roaming animals will be another challenge. In such cases, immunization through either oral route using edible baits incorporating contraceptive immunogen or by dart-gun may provide viable solutions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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